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14. ABSTRACT

The goal of the current grant is to investigate the potential antitumor activity of histone deacetylase inhibitor MS-275 along with the activation of TGFb signaling pathway with the restoration of TGFb receptor II. As presented in our initial proposal, prostate cancer cell line LNCaP has reduced expression in TGFbRII, which is due to the promoter histone deacetylation. Subsequent treatment with chromatin remodeling agent MS-275 was able to restore the expression of TGFbRII. We hypothesized that the restoration of TGFb signaling may contribute to the antitumor activity of MS-275. In the past a year and half, we have focused our effort to identify the re-expression of TGFbRII in vivo, and investigate the antitumor activity of MS-275 in several relevant prostate cancer model. We observed dose and time dependent upregulation of TGFb1 and TGFbRII in LNCaP cells. A cDNA microarray of LNCaP cells (untreated vs. MS-275 treated) has been done, and the data is being analyzed. We observed the antitumor activity of MS-275 in two different models of prostate cancer xenografts. Immunohistochemistry analysis of reactivation of TGFb1 in vivo is on going.

15. SUBJECT TERMS
prostate cancer, Histone deacetylase inhibitors, TGFb signaling

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PROGRESS REPORT.

Introduction

The goal of the current grant is to investigate the potential antitumor activity of histone deacetylase inhibitor MS-275 along with the activation of TGFb signaling pathway with the restoration of TGFb receptor II. As presented in our initial proposal, prostate cancer cell line LNCaP has reduced expression in TGFbRII, which is due to the promoter histone deacetylation. Subsequent treatment with chromatin remodeling agent MS-275 was able to restore the expression of TGFbRII. We hypothesized that the restoration of TGFb signaling may contribute to the antitumor activity of MS-275. In the past a year and half, we have focused our effort to identify the re-expression of TGFbRII in vivo, and investigate the antitumor activity of MS-275 in several relevant prostate cancer model.

Body

Up to date, we have observed the re-expression of TGFbRII in LNCaP in vitro. One of the suppressing finding from these experiments were the identification of upregulation of TGFb1. With physiologically relevant concentrations of MS-275 treatment for 24 hours, both TGFb1 and its receptor TGFbRII were upregulated (Figure 1). This could in part to explain that in vitro combination treatment with MS-275 and TGFb1 did not produce significant amount of additive growth inhibition (Figure 2). Currently, we are optimizing the condition to use neutralizing antibody against TGFb1, hoping to see the reduced growth inhibition, which will indicate the involvement of endogenously activated ligand in our original assay. In terms of the nature of the growth inhibition, we used cell cycle analysis and apoptosis analysis (Figure 3, Table 1) identified that the growth inhibition induced by MS-275 was primarily cell cycle arrest, not apoptosis in LNCaP cells. Similar effect was also observed in another prostate cancer cell line PC-3. In contrast, prostate cancer cell line DU-145 exhibited rapid apoptosis with similar drug concentrations. cDNA microarray analysis of LNCaP cells (treated vs. untreated) has been done, and the data is currently being analyzed.

In vivo mouse model of investigating the antitumor activity of MS-275 has also been conducted. We established LNCaP xenografts in male nude mice. After the tumors were established, mice were randomly distributed into solvent treated control and MS-275 treatment groups, respectively. Daily treatment of MS-275 at 20mg/kg/day effectively inhibited the growth of tumor (Figure 4). Currently, we are optimizing the methods of immunohistochemistry for the purpose of identifying the expression of TGFbRII and TGFb1 in the frozen tumor tissues.

In terms of the impact of MS-275 on the activity of androgen receptor (AR) pathway, we have used prostate cancer cell lines that express PSA, which is a target gene of AR. Treatment of LNCaP and LAPC4 with MS-275 did not alter the expression of PSA expression (Figure 5). More importantly, in vivo, MS-275 did not change the expression of PSA in tumor xenografts of PSA expressing cells either (Figure 5).

To further investigate the antitumor activity of MS-275 and the reactivation of TGFb signaling pathway, we have tried to establish a prostate tumor model that is more relevant to human

prostate cancer. The lethal phenotype of human prostate cancer is the androgen independent prostate cancer metastasis in the bone. We have tried to establish the prostate cancer xenografts in the mouse tibia to mimic the metastatic progression of human prostate cancer. The current difficulty is that all the androgen responsive cell lines grow very poorly in the tibia. We are currently trying to genetically alter the LNCaP cells to increase the tumorigenecity in the bone environment. In contrast, prostate cancer cells PC-3 grew very aggressively in the tibia, and MS-275 treatment were effective in reducing the tumor burden (Figure 6).

Key Research Accomplishment

1. MS-275 upregulated both TGFb1 and TGFbRII expression in LNCaP cells
2. MS-275 was effective in vivo targeting LNCaP growth
3. The growth inhibition of MS-275 was cytostatic with G1 arrest.
4. MS-275 was also effective against prostate cancer cell growth in the bone environment

Reportable Outcome

A manuscript reporting the in vivo antitumor activity of MS-275 in various pre-clinical prostate cancer models is in preparation. We plan to submit it to the journal of prostate in the coming month.

Conclusion

MS-275 was effective against prostate cancer growth in vitro and in vivo. The re-expression of TGFbRII and its ligand TGFb1 in LNCaP cells may be mediators for the MS-275 antitumor activity. However, the upregulation of TGFb1 may be able to promote the tumor microenvironment facilitating tumor growth. Currently, we are optimizing the condition to identify in vivo evidence of the restoration of TGFb signaling pathway in the LNCaP tumors.

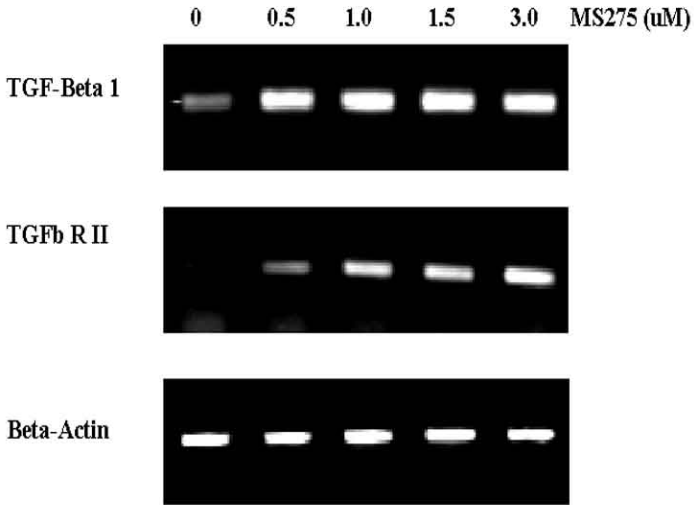

Figure 1 A. Dose-dependent gene upregulation of TGFb1 and TGFbRII in LNCaP cells after MS-275 treatment.

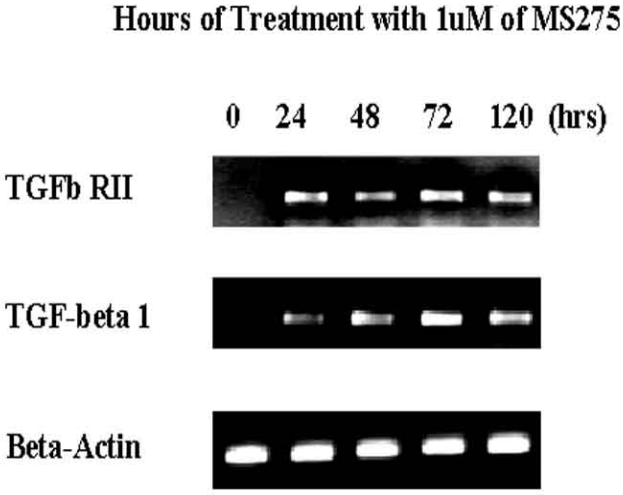

Figure 1B. Time-dependent gene upregulation of TGFb1 and TGFbRII in LNCaP cells after MS-275 treatment.

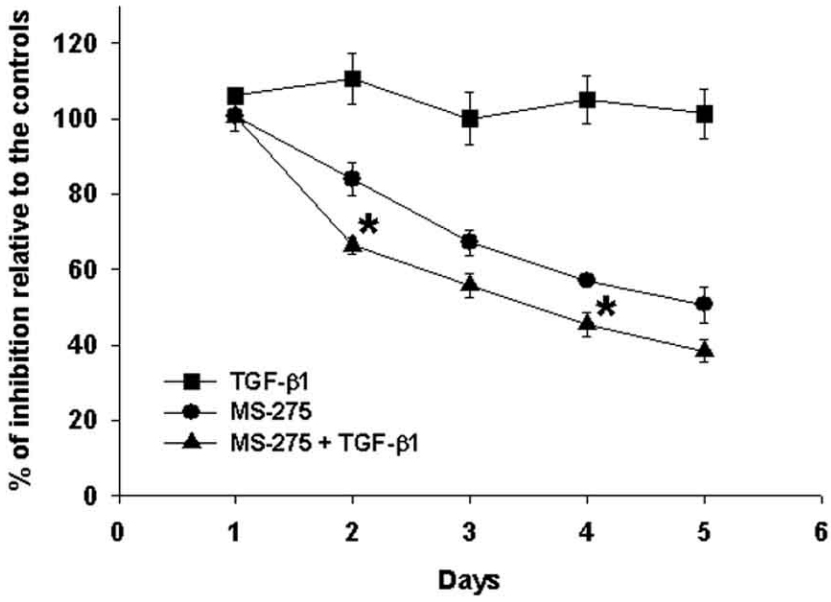

Figure 2. Cell proliferation assay of LNCaP with solvent control, MS-275, TGFb1 only, and as combination. * $P<0.05$ vs. single agent student t test.

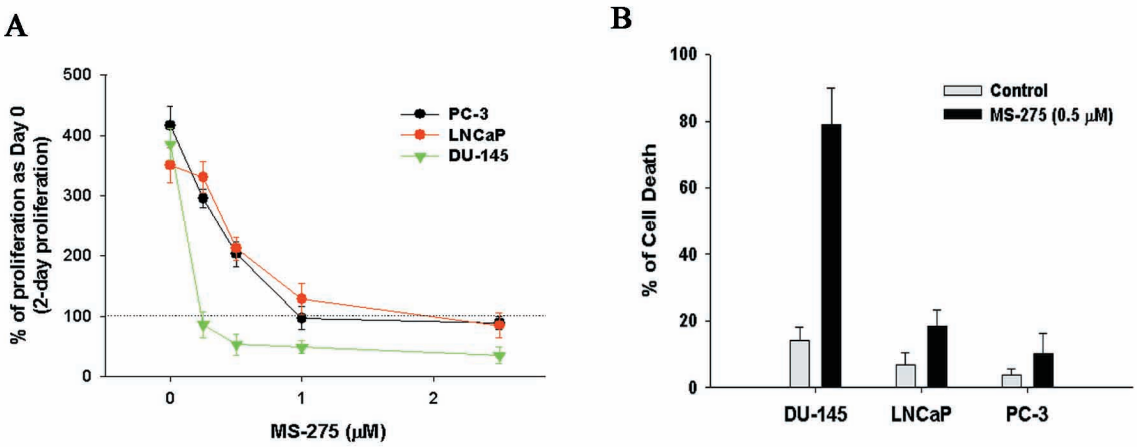

Figure 3. A) PC-3, DU-145 and LNCaP cells were treated with 0, 0.1, 0.5 1.0 and 2.0 μM of MS-275 for 48 hours. The viable cell numbers at Day 2 were measured, and the viable cells numbers at day 0 were normalized to 100%. B) Similar experiments were done as in A) using 0.5 μM of MS-275. Then cells were harvested and stained with Annexin V and PI, and analyzed with FACS.

Table 1. Cell cycle analysis of three prostate carcinoma cell lines with and without MS-275 treatment (0.5 μM for 48 hours). The cell population at each specific stage was expressed as % (control -> treated).

	SubG0 (%)	G0/G1(%)	S (%)	G2/M (%)
DU-145	5 -> 75	55 --> 13	19 --> 9	21 --> 3
LNCaP	1 --> 4	62 --> 73	17 --> 5	20 --> 22
PC-3	7 --> 10	44 --> 52	24 --> 10	25 --> 28

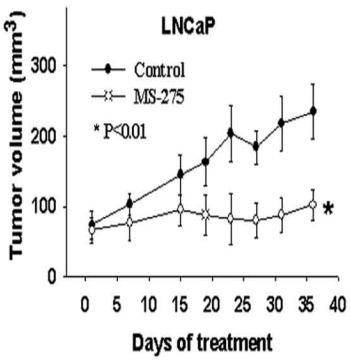

Figure 4. Approximately 2 million LNCaP cells were injected into male nude mice s.c. When the tumors were palpable, daily MS-275 at 20 mg/kg/day were administrated for 5 weeks. Tumor volumes were followed with caliper measurements. Data were mean and SEM, * $P < 0.05$, student t test.

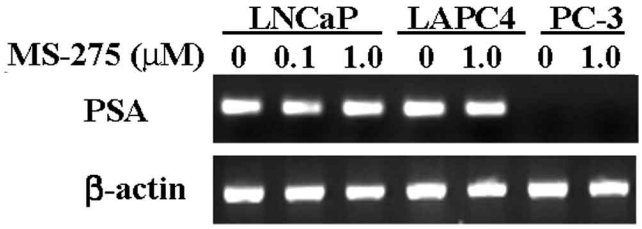

Figure 5. PSA gene expression in LNCaP and LAPC4 cells were analyzed by RT-PCR with and without MS-275. PC-3 cell line that does not express PSA was used as a negative control.

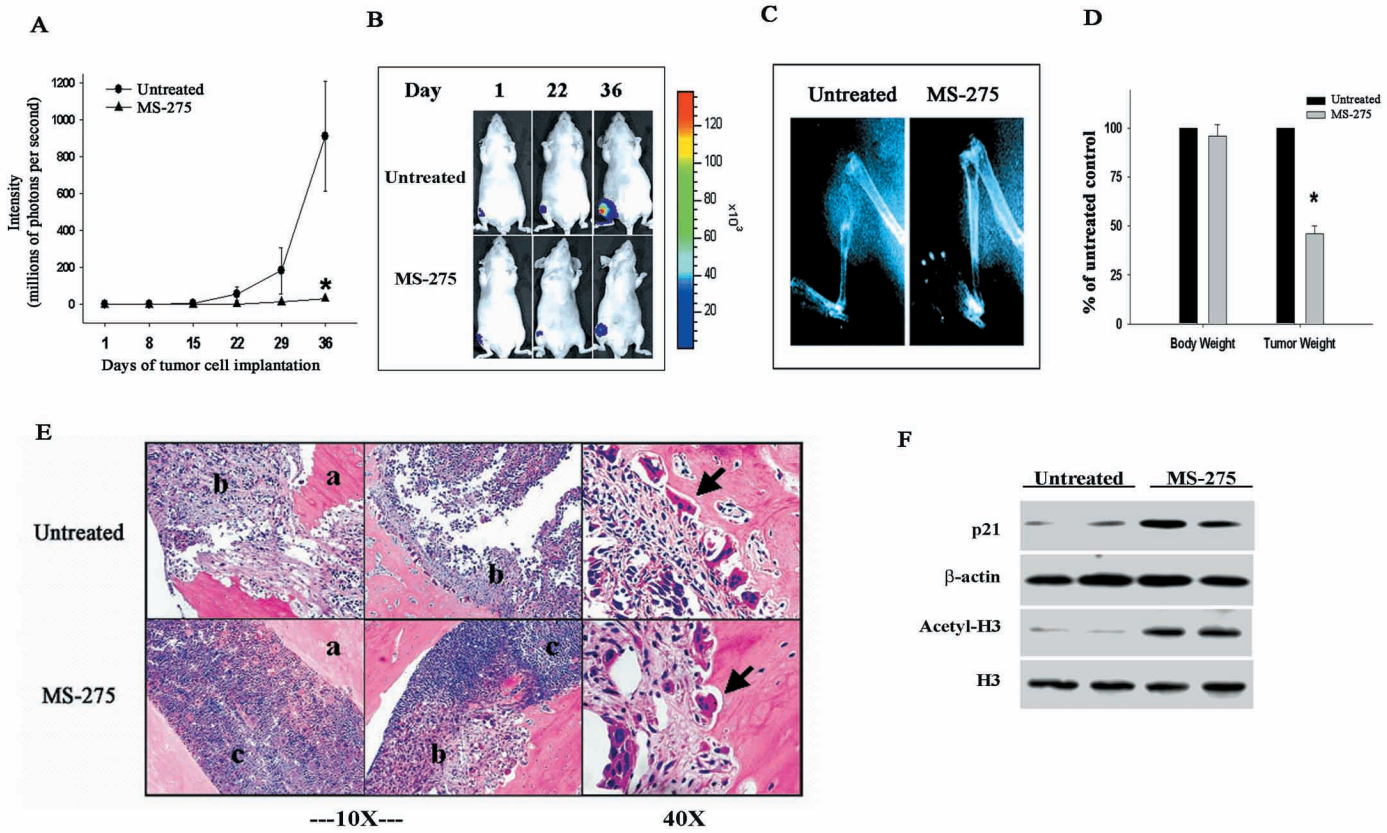

Figure 6. (A) Tumor burden in the control and MS-275 treated groups were followed by in vivo imaging of luciferase activity. The values for each group was expressed as mean and standard error, *, $P < 0.05$. (B) Representative luciferase imaging of one mouse from each group at day 1, 22 and 36 post-injection. (C) Representative radiography of tumor bearing legs in control and MS-275 treated mice at the end of the treatment. (D) The body weight and tumor weight of the mice at the end of the experiment expressed as %of the untreated control group with standard deviation. The net tumor weights were obtained by subtracting the normal contralateral tibia from the tumor-bearing tibia. *, $P < 0.05$. (E) Representative H&E at 10X and 40X magnification. a = bone matrix, b = tumor cells, c = bone marrow cells. Osteoclasts were shown as multinuclear cells indicated by arrow. (F) Western blots of tumor tissue samples showing increased histone H3 acetylation, and p21 expression in MS-275 treated mice.